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REPORT DOCUMENTATION PAGE

DTIC FILE COPY

AD-A203 084

1b. RESTRICTIVE MARKINGS

3. DISTRIBUTION/AVAILABILITY OF REPORT

Approved for public release; distribution unlimited

4. PERFORMING ORGANIZATION REPORT NUMBER(S)

SR88-28

5. MONITORING ORGANIZATION REPORT NUMBER(S)

6a. NAME OF PERFORMING ORGANIZATION: Armed Forces Radiobiology Research Institute

6b. OFFICE SYMBOL (If applicable): AFRRI

7a. NAME OF MONITORING ORGANIZATION

6c. ADDRESS (City, State and ZIP Code): Defense Nuclear Agency, Bethesda, Maryland 20814-5145

7b. ADDRESS (City, State and ZIP Code)

8a. NAME OF FUNDING/SPONSORING ORGANIZATION: Defense Nuclear Agency

8b. OFFICE SYMBOL (If applicable): DNA

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER

8c. ADDRESS (City, State and ZIP Code): Washington, DC 20305

10. SOURCE OF FUNDING NOS.

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT NO.
NWED QAXM			

11. TITLE (Include Security Classification): (see cover)

12. PERSONAL AUTHOR(S): Tolliver, J. M., and Pellmar, T. C.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Yr., Mo., Day)	15. PAGE COUNT
Reprint	FROM _____ TO _____	1988 November	8

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB. GR.	

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT

UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS ☐

21. ABSTRACT SECURITY CLASSIFICATION

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE NUMBER (Include Area Code)	22c. OFFICE SYMBOL
M. E. Greenville	(202) 295-3536	ISDP

DD FORM 1473, 83 APR EDITION OF 1 JAN 73 IS OBSOLETE.

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Brain Research, 456 (1988) 49–56
Elsevier

BRE 13782

Effects of dithiothreitol, a sulfhydryl reducing agent, on CA_1 pyramidal cells of the guinea pig hippocampus in vitro

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(Accepted 9 February 1988)

Key words: Dithiothreitol; Hippocampus; Radioprotectant; Intracellular recording

The radioprotectant, dithiothreitol (DTT) has been shown to increase excitability in the hippocampal slice preparation. In the present study, intracellular recording techniques were used to further examine the actions of DTT. Electrophysiological recordings from CA_1 pyramidal cells were obtained prior to, during and after DTT exposure. DTT caused a small depolarization without altering membrane resistance. DTT induced spontaneous firing and occasional burst firing in normally silent neurons. These effects were accompanied by a reduction in spike frequency adaptation but no change in the afterhyperpolarization following a train of action potentials. Following DTT exposure, orthodromic stimulation produced multiple firing. Subthreshold excitatory postsynaptic potentials (EPSPs) were significantly prolonged. Isolating the CA_1 subfield, attenuated the prolongation of the EPSP by DTT. Recurrent inhibitory postsynaptic potentials were unaffected by DTT. The actions of DTT are likely to result from DTT-induced reduction of disulfide bonds since the reduced form of DTT does not cause a similar hyperexcitability.

INTRODUCTION

The hippocampus is vulnerable to radiation damage. Following exposure to X-radiation, spiking activity appears in electroencephalographic recordings from this area of the brain[12,15,33]. Single unit recordings in vivo show that neuronal firing patterns in the hippocampus are disrupted by radiation[2]. In vitro studies indicate that γ-radiation can impair both synaptic and extrasynaptic mechanisms in the hippocampus[41].

Dithiothreitol (DTT), a disulfide reducing agent[4], has been used as a radioprotectant in cellular and enzyme systems[17,18,31,32]. It is thought to act both by scavenging free radicals[18,31,32] and by donating hydrogen to damaged macromolecules[18]. Many studies have demonstrated that DTT affects neurotransmitter systems including acetylcholine[3,16,21,25,30] dopamine[37], opiates[27], norepinephrine[24,28] and histamine[10,11]. All these transmitters are effective agents in the hippocampal slice (for review see ref. 6). A previous study showed that DTT increases hippocampal excitability, causing spontaneous and evoked burst firing[40]. Both synaptic and extrasynaptic mechanisms were implicated. The present study uses intracellular recording techniques to examine the mechanism(s) whereby DTT increases neuronal excitability. Preliminary results have been presented elsewhere[39].

MATERIALS AND METHODS

Hippocampal slices (400–450 μm thick) were prepared from euthanized male Hartley guinea pigs as previously described[29,40]. Slices were incubated at room temperature in oxygenated solution (see below) for at least 2 h to allow recovery from the dissection. One slice was then placed in a submerged slice chamber (Zbicz design)[44]. The tissue was continuously superfused (0.8–1.0 ml/min) with a solution

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containing (in mM): NaCl 124, KCl 3.0, $CaCl_2$ 2.4, $MgSO_4$ 1.3, KH_2PO_4 1.24, $NaHCO_3$ 26.0 and glucose 10.0, oxygenated with 95% O_2/5% CO_2 and maintained at 30° ± 1 °C.

DTT, obtained from Calbiochem (Lot numbers 233153 and 410163), was dissolved in the bathing solution immediately prior to use to give a final concentration of 0.5 mM. After obtaining control data, the slices were superfused with the DTT solution for 25 min. This dose and exposure time were chosen because they consistently increased excitability in the field potential recordings. Subsequent to DTT exposure, slices were superfused with normal solution for the remainder of the experiment. Data were collected for a minimum of 60 min after the initial exposure to DTT.

Concentric, bipolar stainless steel electrodes were used to provide constant-current stimuli (up to 0.5 mA, 200 μs duration, 0.2 Hz) to hippocampal pathways. Cells in the CA_1 region of hippocampus were orthodromically activated by stimulation of afferent fibers in the stratum radiatum. Antidromic potentials were elicited by stimulation of the alveus.

Intracellular recordings from CA_1 pyramidal cells were obtained through electrodes filled with either 2 M KCl (20–40 MΩ) or 4 M potassium acetate (70–100 MΩ). A conventional bridge circuit (Dagan 8100) allowed potential recording and intracellular current injection via the same electrode. The bridge was balanced frequently by passing pulses of current and monitoring the potential on the oscilloscope. Data were recorded on a Gould chart recorder and a Tektronix oscilloscope, and were digitized and stored on an LSI 11-03 minicomputer. In all experiments, somatic field potentials were monitored concurrently using electrodes (1–10 MΩ) filled with 2 M NaCl. Field potentials were amplified using a high-gain differential preamplifier. They were either photographed directly off the oscilloscope or digitized, and stored on an LSI 11-03 minicomputer.

The effects of DTT on the following electrophysiological parameters were examined: membrane potential, input resistance, spike frequency adaptation, afterhyperpolarization (AHP), excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs). Membrane resistance was calculated from the membrane potential change produced by injections of 0.25 or 0.50 nA hyperpolarizing current. To study spike frequency adaptation, suprathreshold depolarizing current pulses of 700 ms duration were applied via the recording electrode at 0.05–0.1 Hz. An AHP was elicited every 10–20 s by injecting a train of 4 suprathreshold depolarizing current pulses (8 ms duration, 80 Hz). EPSPs were evoked by stimulation (10–75 μA) of the stratum radiatum and recurrent IPSPs by stimulation (25–100 μA) of the alveus. In several experiments the CA_1 region was isolated; CA_2 and CA_3 were removed by cutting the slice with a scalpel blade. No attempt was made to verify histologically that all of the CA_2 and CA_3 region was removed in these experiments. The slice was allowed to recover from the isolation procedure for at least 45 min before attempting to impale CA_1 neurons.

RESULTS

As previously reported[40], field potential recordings in field CA_1 of hippocampus revealed an increase in excitability following exposure to 0.5 mM DTT for 25 min. Twenty to 40 min after initial exposure to DTT, the baseline 'noise' level increased in the extracellular recordings from stratum radiatum. This was followed by an increase in the amplitude of the orthodromic population spike and the emergence of multiple (3–7) peaks in the orthodromic field potential (Fig. 1A). This effect was sustained for the duration (1–2 h) of the experiment.

The increased excitability produced by DTT was further examined in CA_1 pyramidal cells with intracellular recording techniques. Prior to drug exposure, CA_1 pyramidal cells were generally silent; spontaneous action potentials were rare. Twenty to 40 min following initial exposure to 0.5 mM DTT, spontaneous activity was always evident (Fig. 1B). This effect coincided with the appearance of 'noise' in the extracellular recording. In approximately 60% of the cells, spontaneous doublets and burst firing occurred in an irregular pattern. Similar spontaneous bursting activity was observed in field potential recordings[40].

The intracellular changes were accompanied by a small, but statistically significant depolarization of the membrane potential. The mean (± S.E.M.) resting potential of all 25 cells before exposure to DTT was −64.6 ± 0.8 mV. Sixty minutes after initial expo-

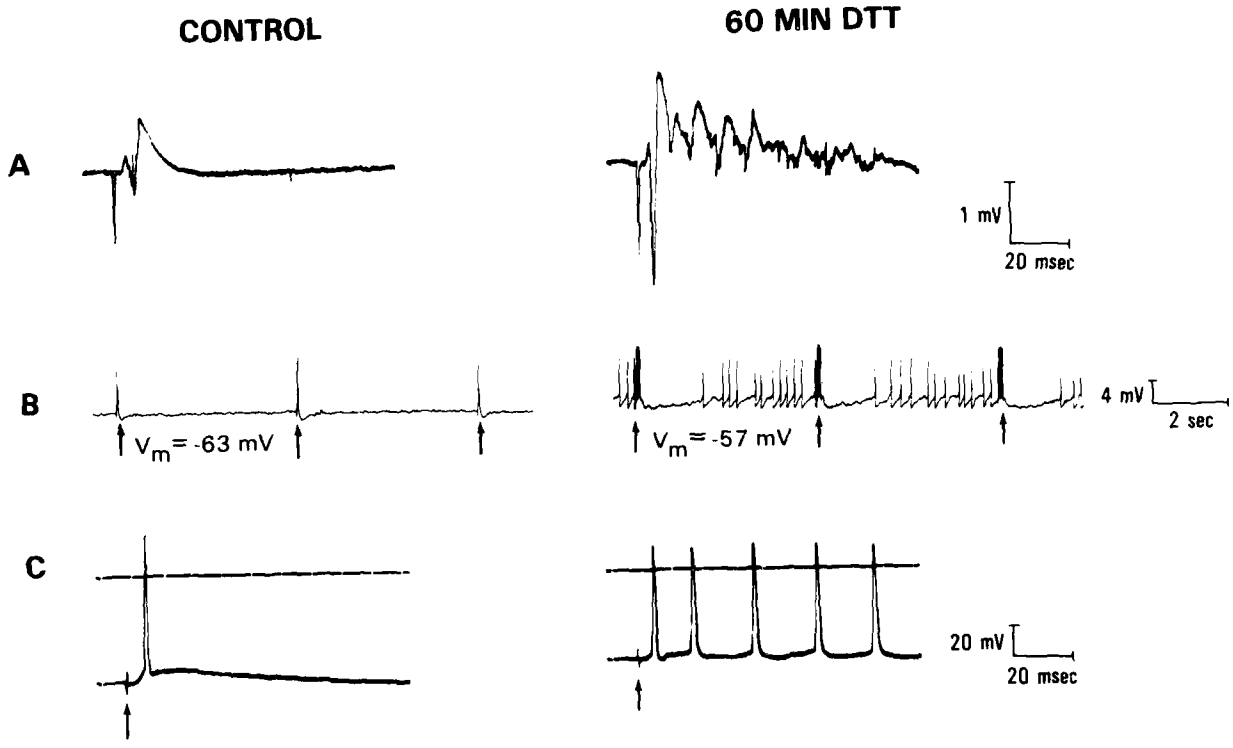

Fig. 1. DTT altered the orthodromic activity in CA_1 of the hippocampal slice. A: orthodromic field potentials in response to stimulation of the stratum radiatum prior to and 60 min after beginning exposure to DTT (0.5 mM). DTT increased the amplitude of the initial population spike and caused multiple spiking. B: DTT induced spontaneous action potential firing, increased synaptic input and caused some membrane depolarization. At the arrows, stratum radiatum was stimulated. Action potentials were truncated by the chart recorder. C: orthodromic action potentials recorded from CA_1 pyramidal cell. Straight line indicates zero potential. The onset of multiple spiking in the orthodromic field potential coincided with the appearance of multiple firing recorded intracellularly.

sure to DTT the membrane potential was depolarized to -61.6 ± 0.7 mV (Student's *t*-test for paired samples, $n = 25$, $P < 0.05$). DTT did not significantly alter the input resistance of CA_1 pyramidal cells. The input resistance in control was 41.7 ± 4.7 MΩ and following exposure to DTT was 44.0 ± 5.0 MΩ ($n = 12$, $P > 0.05$).

Increased excitability of CA_1 pyramidal cells was evident when they were stimulated orthodromically. In control, the stimulus applied to the stratum radiatum was adjusted to elicit an EPSP with a single action potential (Fig. 1C, Control). After exposure to DTT, the same orthodromic stimulus evoked multiple (3–7) action potentials (Fig. 1C). The onset of multiple firing of pyramidal cells in response to orthodromic stimulation coincided with the appearance of multiple spiking in the orthodromic field potential (Fig. 1A, C). Even when membrane potential was maintained at pre-DTT levels, multiple spikes were elicited by orthodromic stimulation. Subthreshold EPSPs were examined in 5 cells. Comparisons of EPSPs before and after DTT exposure were always made at the same membrane potential. The rate of rise and the amplitude of the early component of the EPSP were not significantly affected by DTT (Fig. 2A). The mean amplitude in control was 6.2 ± 0.5 mV while after exposure to DTT was 5.9 ± 0.6 mV ($n = 5$, $P > 0.05$). The rate of rise of the EPSP was 1.4 ± 0.2 mV/ms in control and 1.5 ± 0.2 mV/ms after DTT exposure ($n = 5$, $P > 0.05$). Similarly, the extracellularly recorded population synaptic response showed no change in the initial slope[40]. DTT did, however, consistently, prolong the EPSP (Fig. 2A). DTT increased the mean duration from 40.5 ± 4.4 to 91.4 ± 13.3 ms ($n = 4$, $P < 0.05$) measured from the initial depolarization to the time it returned to the resting membrane potential. The shape of the EPSP depended on stimulus strength. Stimulation with very low currents could evoke only a delayed depolarizing potential with no initial EPSP. As the stim-

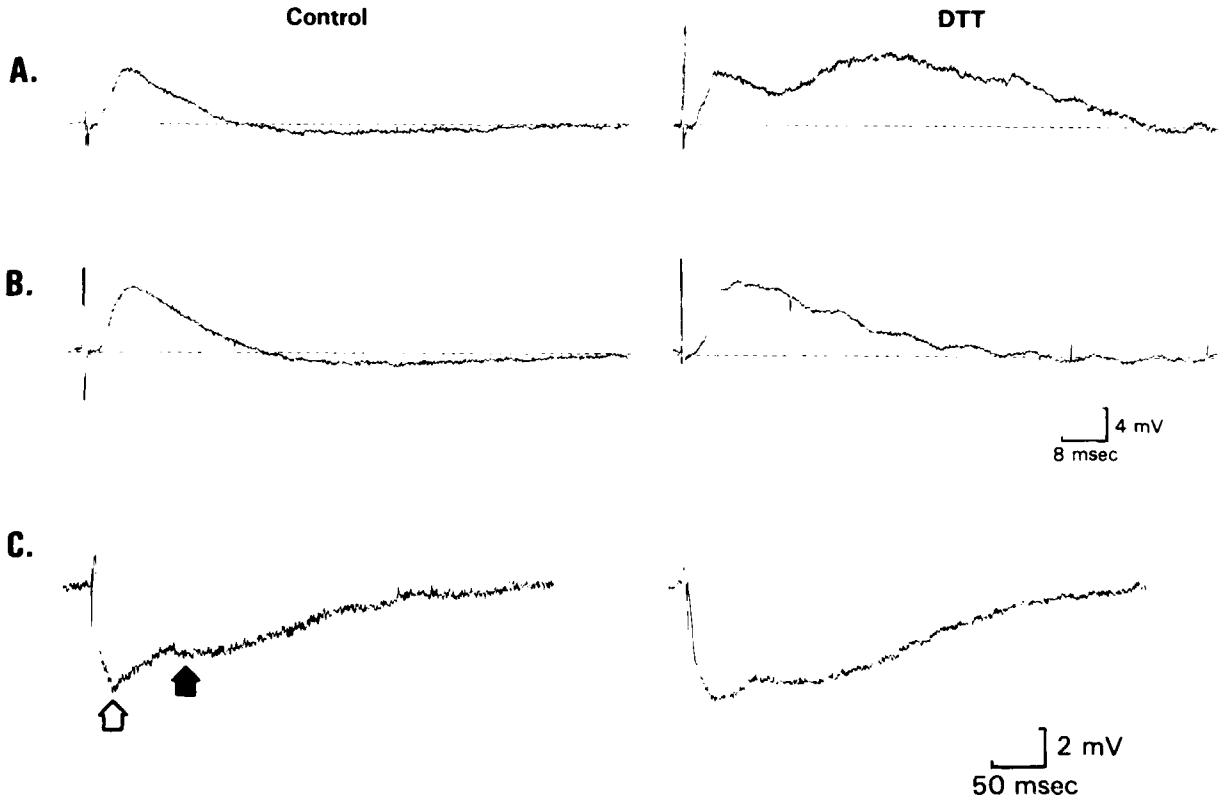

Fig. 2. DTT prolonged the EPSP but did not block recurrent inhibition. A: EPSPs recorded from a CA_1 pyramidal cell prior to and 45 min after starting superfusion with 0.5 mM DTT. DTT had little effect on the rate of rise or the amplitude of the EPSP. The EPSP was, however, prolonged. Both traces were recorded at a membrane potential of −68 mV. B: EPSPs recorded from a pyramidal cell located in the isolated CA_1 region. EPSP prior to DTT exposure (control) and 45 min after initial exposure to DTT. DTT was less effective in prolonging the EPSP in the isolated CA_1 region than in the intact slice. Traces in B were recorded at a membrane potential of −63 mV. C: recurrent IPSPs were elicited in CA_1 pyramidal cell by subthreshold stimulation of the alveus. DTT (0.5 mM) did not block the early (open arrow) or late (filled arrow) phase of the IPSP. IPSP was measured with membrane potential at −60 mV.

ulus strength was increased, both the initial EPSP and the delayed depolarizing potential became larger. With further increases in stimulus strength, the EPSP and the later depolarizing potential merged together to form the prolonged EPSP. At sufficient stimulus strength, the longer latency depolarizing potential reached threshold, resulting in action potential firing.

Stimulation of stratum radiatum might antidromically activate CA_2/CA_3, which could cause late synaptic activation of CA_1 pyramidal cells, and thus prolong the EPSP. To examine this possibility, the effects of DTT on the EPSP were examined in slices in which CA_1 was isolated by removal of CA_2/CA_3. Slices were allowed to recover for at least 45 min before intracellular recording. Cells in the isolated sections did not show any signs of injury. Resting membrane potential (-62.4 ± 1.2 mV, $n = 3$) and membrane resistance (44.7 ± 5.4 MΩ, $n = 3$) were not significantly different from control values in intact slices. In the isolated CA_1 regions, DTT increased the EPSP duration from 49.3 ± 6.6 to 69.6 ± 11.8 ms ($n = 3$, $P < 0.05$) (Fig. 2B). Two of the 3 cells tested showed a minimal increase in duration (40–54.3 ms and 40–51.4 ms). The EPSP of the third cell, however, was greatly prolonged (68–103 ms). These results contrasted with those of the intact slice where the EPSP of all 5 cells showed a substantial increase in duration. The variability observed in the isolated sections may be due to the degree of isolation of CA_1 actually achieved.

DTT might also increase excitability by decreasing recurrent inhibition. IPSPs were elicited by subthreshold stimulation of the alveus. Despite the ap-

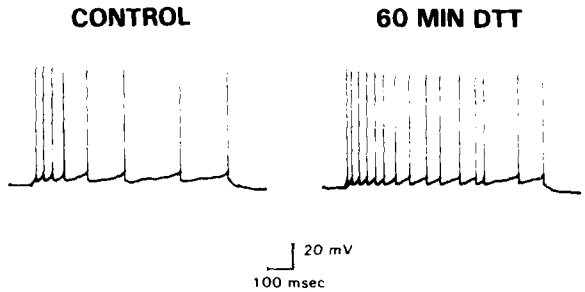

Fig. 3. DTT decreased spike frequency adaptation recorded from CA_1 pyramidal cell. Spike frequency adaptation was examined by stimulating a cell with depolarizing current pulses of 700 ms duration. Recordings were made prior to (Control) and 60 min after starting DTT exposure. Membrane potential remained at −65 mV.

pearance of increased excitability, DTT did not reduce the amplitude of the early or the late phase of the recurrent IPSP (early IPSP, control 5.4 ± 0.6 mV, DTT 5.2 ± 0.5 mV; late IPSP, control 2.3 ± 0.6 mV, DTT 2.1 ± 1.3 mV, $n = 3$, $P > 0.05$) (Fig. 2C). The duration of the IPSP also did not change following exposure to DTT (control, 319.2 ± 38.6 ms; DTT, 301.8 ± 60.0 ms, $n = 3$, $P > 0.05$).

A depolarizing current pulse of 700 ms duration produced a train of action potentials that decreased in frequency during the step (Fig. 3). This phenomenon, spike frequency adaptation, has been well described in hippocampal neurons[26]. Spike frequency adaptation was examined prior to and after DTT exposure in 4 cells (Fig. 3). DTT decreased spike frequency adaptation in all cells examined. DTT more than doubled (2.29 ± 0.25 times, $n = 3$) the number of action potentials elicited by 700 ms depolarizing step. The increased number of action potentials did not result from a change in the resting membrane potential or input resistance. There was no obvious change in the shape of the evoked action potentials.

Spike frequency adaptation is, in part, due to the temporal summation of the calcium-dependent slow AHP. The effects of DTT on the AHP produced by a train of 4 action potentials were examined in 4 cells. As shown in Fig. 4, DTT did not alter the early or late phase of the AHP. In control, the late AHP was 6.75 ± 1.60 mV while after exposure to DTT, the AHP was 6.38 ± 1.55 mV ($n = 4$, $P > 0.05$).

DISCUSSION

This study demonstrates that DTT increases the excitability of CA_1 pyramidal cells in the hippocampal slice. This increased excitability is manifested by the appearance of the following phenomena recorded from CA_1 pyramidal cells: (1) spontaneous spiking and occasional spontaneous bursting activity; (2) abnormal repetitive firing in response to orthodromic stimulation; and (3) a reduction in spike frequency adaptation. The appearance of these intracellular events coincides with the onset of changes in the orthodromic field potential recordings reported previously[40].

A prolongation of the EPSP appears to be responsible for the repetitive firing elicited by orthodromic stimulation following treatment with DTT. One mechanism that could account for the increased EPSP duration is the blockade of inhibitory inputs to the pyramidal cells. The convulsants bicuculline and penicillin prolong the EPSP through this action[6–9,34]. In contrast to these agents DTT does not alter the recurrent IPSP. Feedforward inhibition was not tested, however, and may be affected by DTT.

An alternative mechanism for EPSP prolongation is inhibition of the reuptake of the excitatory neurotransmitter. The transmitter released by Schaffer collaterals at the CA_1 pyramidal cells is thought to be the excitatory amino acid, glutamate[5,36]. Considering that DTT (0.5 mM) decreases γ-aminobutyric acid reuptake[20], it is feasible that through a similar mech-

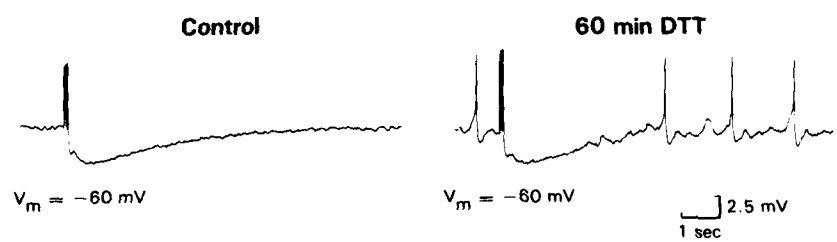

Fig. 4. DTT did not alter the early or the late phase of the AHP following 4 action potentials evoked by a train of 4 suprathreshold current pulses (8 ms, 80 Hz). Traces were recorded prior to and 60 min following the start of DTT superfusion.

anism, DTT could impair the reuptake of excitatory amino acids. The multiphasic shape of the prolonged EPSP following DTT exposure is inconsistent with this possibility.

A third mechanism for prolongation of the EPSP is that DTT alters the interaction of the CA_1 and the CA_2/CA_3 subfields. Stimulation of the stratum radiatum to elicit an orthodromic response in CA_1 also antidromically activates the CA_2/CA_3 region. If the excitability of this region was elevated by DTT, then stimulation of the stratum radiatum could result in a reverberating circuit between CA_1 and the CA_2/CA_3 subfields. Thus a reactivation of CA_1 resulting from the delayed firing of the CA_2/CA_3 region would prolong the EPSP[14,43]. Support for this hypothesis comes from the observation that DTT seems to be less effective in prolonging the EPSP in isolated CA_1 regions than in intact slices. In addition, it was possible to stimulate the stratum radiatum at a sufficiently low stimulus strength to evoke a long latency potential in the absence of an early EPSP. This longer latency potential most likely represents activation of CA_1 resulting from the delayed firing of the CA_2/CA_3 region in response to the stimulation of the stratum radiatum.

DTT probably does not increase excitability of CA_1 pyramidal cells by increasing transmitter release or by enhancing sensitivity of the receptor mediating normal synaptic excitation. If these mechanisms were responsible for the increased excitability, DTT would be expected to increase the amplitude and rate of rise of the EPSP. Instead, DTT had minimal effects on these parameters. Although the neurotransmitter mediating the EPSP produced by Schaffer collateral stimulation is uncertain, it is likely to be glutamate[5,36]. To date, no effects of DTT on the excitatory amino acid receptors have been reported. DTT does not alter the response of *Onchidium* esophageal ganglia to applied L-glutamate[22]. Other sulfhydryl modifying reagents such as mercuric chloride, *n*-chloromercuribenzoate and *n*-ethylmaleimide depress rather than enhance glutamate and kainate responses recorded from pyramidal neurons of the rat hippocampus[23].

Extracellular recordings suggested that DTT increased excitability through both synaptic and extrasynaptic mechanisms[40]. Most neurons were somewhat depolarized following exposure to DTT. Similarly Terrar[38] observed that a 30-min exposure to DTT caused a small depolarization in muscle. Such a mechanism is not likely to explain all the actions of DTT, however. The oxidized form of DTT (*trans*-4,5 dihydroxy-1,2 dithiane, OxDTT) was found in extracellular experiments to be ineffective in inducing multiple spiking in the orthodromic field potential[40]. Preliminary intracellular experiments indicate that, although OxDTT causes a similar depolarization (3–8 mV), it does not induce burst firing.

DTT decreased spike frequency adaptation. This effect would promote repetitive firing since normal mechanisms to attenuate trains of actions potentials are impaired. The calcium-dependent potassium current contributes significantly to regulation of firing frequency in hippocampal pyramidal cells[26]. A decrease in this current, however, is unlikely to be the mechanism since the AHP (resulting from the calcium-dependent potassium current[1,13,19,35,42]) is unaffected by DTT.

In conclusion, DTT increases the excitability of the hippocampus in vitro. This effect probably results from DTT-induced reduction of disulfide bonds since the reduced form, OxDTT, does not cause similar hyperexcitability. DTT is known to interact with a number of neurotransmitter systems which are present in the hippocampus. Although stimulation of stratum radiatum predominantly activates the Schaffer collaterals and therefore an excitatory amino acid pathway, other inputs and other transmitter systems are likely to be activated. It is not unlikely that alteration of these systems would contribute to the dysfunction of the hippocampus following DTT exposure. The abnormal activity produced by DTT in neural tissue would limit its usefulness as a radioprotectant.

ACKNOWLEDGEMENTS

Supported by the Armed Forces Radiobiology Research Institute, Defense Nuclear Agency, under work unit 00105. Views presented in this paper are those of the authors; no endorsement by the Defense Nuclear Agency has been given or should be inferred. Research was conducted according to the principles enunciated in the 'Guide for the Care and Use of Laboratory Animals' prepared by the Institute of Laboratory Animal Resources, National Research Council.

REFERENCES

1 Alger, B.E. and Nicoll, R.A., Epileptiform burst afterhyperpolarization: a calcium dependent potassium potential in hippocampal CA1 pyramidal cells, *Science*, 210 (1980) 1122–1124.

2 Bassant, M. and Court, L., Effect of whole-body irradiation on the activity of rabbit hippocampal neurons, *Radiat. Res.*, 75 (1978) 593–606.

3 Brown, D.A. and Kwiatkawski, D., A note on the effect of dithiothreitol (DTT) on the depolarization of isolated sympathetic ganglia by carbachol and bromoacetylcholine, *Br. J. Pharmacol.*, 56 (1976) 128–130.

4 Cleland, W.W., Dithiothreitol. A new protective reagent for SH groups, *Biochemistry*, 3 (1964) 480–482.

5 Cotman, C.W. and Nadler, J.V., Glutamate and aspartate as hippocampal transmitters: biochemical and pharmacological evidence. In P.J. Roberts, J. Storm-Mathisen and G.A.R. Johnson (Eds.), *Glutamate: Transmitter in the Central Nervous System*, Wiley and Sons, New York, 1981, pp. 117–154.

6 Dingledine, R., Hippocampus: synaptic pharmacology. In R. Dingledine (Ed.), *Brain Slices*, Plenum, New York, 1984, pp. 87–112.

7 Dingledine, R. and Gjerstad, L., Penicillin blocks hippocampal i.p.s.p.s unmasking prolonged e.p.s.p.s, *Brain Research*, 168 (1979) 205–209.

8 Dingledine, R. and Gjerstad, L., Reduced inhibition during epileptiform activity in the in vitro hippocampal slice, *J. Physiol. (Lond.)*, 305 (1980) 297–313.

9 Dingledine, R., Hynes, M.A. and King, G.L., Involvement of *N*-methyl-D-aspartate receptors in epileptiform bursting in the rat hippocampal slice, *J. Physiol. (Lond.)*, 380 (1986) 175–189.

10 Donaldson, J. and Hill, S.J., Enhancement of histamine N_1-receptor agonist activity by 1,4-dithiothreitol in guinea-pig cerebellum and cerebral cortex, *J. Neurochem.*, 47 (1986) 1476–1482.

11 Donaldson, J. and Hill, S.J., 1,4-Dithiothreitol-induced alteration in histamine H_1-agonist binding in guinea-pig cerebellum and cerebral cortex, *Eur. J. Pharmacol.*, 129 (1986) 25–31.

12 Gangloff, H., Acute effects of X-irradiation on brain electrical activity in cats and rabbits. In *Effects of Ionizing Radiation on the Nervous System*, International Atomic Energy Agency, Vienna, 1962, pp. 123–135.

13 Gustafsson, B. and Wigstrom, H., Evidence for two types of afterhyperpolarization in CA1 pyramidal cells in the hippocampus, *Brain Research*, 206 (1981) 462–468.

14 Hablitz, J.J., Picrotoxin-induced epileptiform activity in hippocampus: role of endogenous versus synaptic factors, *J. Neurophysiol.*, 51 (1984) 1011–1027.

15 Haley, T.J., Changes induced in brain activity by low doses of X-irradiation. In *Effects of Ionizing Radiation on the Nervous System*, International Atomic Energy Agency, Vienna, 1962, pp. 123–135.

16 Hedlund, B. and Bartfai, T., The importance of thiol and disulfide groups in agonist and antagonist binding to the muscarinic receptor, *Mol. Pharmacol.*, 15 (1979) 531–544.

17 Held, K.D., Interactions of radioprotectors and oxygen in cultured mammalian cells. I. Dithiothreitol effects on radiation-induced cell killing, *Radiation Res.*, 101 (1985) 424–433.

18 Held, K.D., Harrop, H.A. and Michael, B.D., Effects of oxygen and sulfhydryl-containing compounds on irradiated transforming DNA. II. Glutathione, cysteine, and cysteamine, *Int. J. Radiat. Biol.*, 45 (1984) 615–626.

19 Hotson, J.R. and Prince, D.A., A calcium-activated hyperpolarization follows repetitive firing in hippocampal neurons, *J. Neurophysiol.*, 43 (1980) 409–419.

20 Iversen, L.L. and Johnston, G.A.R., GABA uptake in rat central nervous system: comparison of uptake in slices and homogenates and the effects of some inhibitors, *J. Neurochem.*, 18 (1971) 1939–1952.

21 Karlin, A. and Bartels, E., Effects of blocking sulphydryl groups and of reducing disulphide bonds on the acetylcholine-activated permeability system of the electroplax, *Biochim. Biophys. Acta*, 126 (1966) 525–535.

22 Kato, M., Oomura, Y. and Maruhashi, J., Effects of chemical modification on the L-glutamate receptors on the *Onchidium* neurons, *Jap. J. Physiol.*, 33 (1983) 535–546.

23 Kiskin, N.I., Krishtal, O.A., Tsyndrenko, A.Ya. and Akaike, N., Are sulfhydryl groups essential for function of the glutamate-operated receptor–ionophore complex, *Neurosci. Lett.*, 66 (1986) 305–310.

24 Lucas, M., Hanoune, J. and Bockaert, J., Chemical modification of the beta adrenergic receptor coupled with adenylate cyclase by disulfide bridge-reducing agents, *Mol. Pharmacol.*, 14 (1978) 227–236.

25 Lukas, R.J. and Bennett, E.L., Chemical modification and reactivity of sulfhydryls and disulfides of rat brain nicotinic-like acetylcholine receptors, *J. Biol. Chem.*, 255 (1980) 5573–5577.

26 Madison, D.V. and Nicoll, R.A., Control of the repetitive discharge of rat CA1 pyramidal neurones in vitro, *J. Physiol. (Lond.)*, 354 (1984) 319–331.

27 Marzullo, G. and Hine, B., Opiate receptor function may be modulated through an oxidation-reduction mechanism, *Science*, 208 (1980) 1171–1173.

28 Pederson, S.E. and Ross, E.M., Functional activation of beta-adrenergic receptors by thiols in the presence or absence of agonists, *J. Biol. Chem.*, 260 (1985) 14150–14157.

29 Pellmar, T.C., Electrophysiological correlates of peroxide damage in guinea pig hippocampus in vitro, *Brain Research*, 364 (1986) 377–381.

30 Rang, H.P. and Ritter, J.M., The effects of disulphide bond reduction on the properties of cholinergic receptors in chick muscle, *Mol. Pharmacol.*, 7 (1971) 620–631.

31 Redpath, J.L., Pulse radiolysis of dithiothreitol, *Radiation Res.*, 54 (1973) 364–374.

32 Redpath, J.L., Radioprotection of enzyme and bacterial systems by dithiothreitol, *Radiation Res.*, 55 (1973) 109–117.

33 Schoenbrun, R.L., Campeau, E. and Adey, W.R., Electroencephalographic and behavioral effects from x-irradiation of the hippocampal system. In T.J. Haley and R.S. Snider (Eds.), *Response of the Nervous System to Ionizing Radiation, Second International Symposium*, Little and Brown, Boston, 1964, pp. 411–428.

34 Schwartzkroin, P.A. and Prince, D.A., Changes in excitatory and inhibitory potentials leading to epileptogenic activity, *Brain Research*, 183 (1980) 61–76.

35 Schwartzkroin, P.A. and Stafstrom, C.E., Effects of EGTA on the calcium-activated afterhyperpolarization in hippocampal CA3 pyramidal cells, *Science*, 210 (1980) 1125–1126.

36 Storm-Mathisen, J., Localization of transmitter candidates in the brain: the hippocampal formation as a model, *Prog.*

Neurobiol., 8 (1977) 119–181.
37 Suen, E.T., Stefanini, E. and Clement-Cormier, Y.C., Evidence for essential thiol groups and disulfide bonds in agonist and antagonist binding to the dopamine receptor, *Biochem. Biophys, Res. Commun.*, 96 (1980) 953–960.
38 Terrar, D.A., Effects of dithiothreitol on end-plate currents, *J. Physiol. (Lond.)*, 276 (1978) 403–417.
39 Tolliver, J.M. and Pellmar, T.C., Intracellular analysis of dithiothreitol effects on guinea pig CA1 pyramidal cells, *Soc. Neurosci. Abstr.*, 12 (1986) 676.
40 Tolliver, J.M. and Pellmar, T.C., Dithiothreitol elicits epileptiform activity in CA1 of the guinea pig hippocampal slice, *Brain Research*, 404 (1987) 133–141.
41 Tolliver, J.M. and Pellmar, T.C., Ionizing radiation alters neuronal excitability in hippocampal slices of the guinea pig, *Radiation Res.*, 112 (1987) 555–563.
42 Wong, R.K.S. and Prince, D.A., Afterpotential generation in hippocampal pyramidal cells, *J. Neurophysiol.*, 45 (1981) 86–97.
43 Wong, R.K.S. and Traub, R.D., Synchronized burst discharge in disinhibited hippocampal slice. I. Initiation in CA2/CA3 region, *J. Neurophysiol.*, 49 (1983) 442–458.
44 Zbicz, K.L. and Weight, F.F., Transient voltage and calcium dependent outward currents in hippocampal CA3 pyramidal neurons, *J. Neurophysiol.*, 40 (1985) 1038–1058.